

(19) World Intellectual Property Organization
International Bureau(43) International Publication Date
12 July 2001 (12.07.2001)

PCT

(10) International Publication Number
WO 01/49336 A1(51) International Patent Classification⁷: A61L 27/52. 31/14, 27/16, 31/04

(81) Designated States (national): AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW.

(21) International Application Number: PCT/IT99/00428

(22) International Filing Date:
29 December 1999 (29.12.1999)

(25) Filing Language: English

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

(71) Applicant (for all designated States except US): PROGEN S.R.L. [IT/IT]; Corso Umberto I, 141, I-72100 Brindisi (IT).

Published:

— With international search report.

(72) Inventors; and

(75) Inventors/Applicants (for US only): LOPATINE, Vladislav [RU/RU]; ul. Frunzenskaja 3-77, Moscow 119270 (RU). MATIACHVILI, Grigori [RU/RU]; ul. Krasnoarmeyskaja 12-6, Moscow 125167 (RU).

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(74) Agent: LANZONI, Luciano; Bugnion S.p.A., Via Goito, 18, I-40126 Bologna (IT).

WO 01/49336 A1

Check against next WO &

Filed 29 Dec 1999 6/4

11/29/2000

in 29/11/2000

Pub date is not art date
12 July 2001 is pub dateArt is not
pub date

(54) Title: BIOCOMPATIBLE HYDROGEL AND METHOD OF ITS PRODUCTION

(57) Abstract: The present invention relates to a composition and a method of producing a biologically compatible hydrogel for endoprosthetics containing cross-linked copolymer of acrylamide with a linking agent and water wherein said linking agent can be a mixture of acrylamide, N, N'-methylene-bis-acrylamide, N, N'-ethylene-bis-acrylamide, ethylene-bis-(oxyethylene nitril)-tetracetic oxide or ethylene-bis-(oxyethylene nitril)-tetracetic acid.

DescriptionBIOCOMPATIBLE HYDROGEL AND METHOD OF ITS PRODUCTION5 Technical Field

The invention relates to a composition and a method of producing a biologically compatible hydrogel on the basis of cross-linked copolymer of acrylamide with a linking agent, which can be used as medical purpose material, 10 e.g.:

for endoprosthetics by means of purposeful injection of hydrogel. For plastics of soft facial tissue, mammal gland, penis, sural muscle, cords and other tissues fitting hydrogel consistence;

15 as a filling agent for endoprostheses including mammal gland endoprostheses;

as medicine depot for protracted medication of tumors and abscesses (for example).

Background Art

20 The task of obtaining synthetic materials for soft tissue (muscles and subcutaneous tissue) substitutions is essential for medical practice. These materials must be relatively cheap and easy to produce. They must have necessary physicochemical properties (such as certain consistence and chemical 25 inertness, capability to shrink and swell after being placed into the body) as well as biological properties (biological inertness, lack of rejection or any other tissue response). Besides, a material must have a convenient form for muscular tissue injection with a minimal injury of patient's body.

Acrylamide-based hydrogels can be used as such a material. GB Patent 30 No 2114578 describes hydrogel on the basis of copolymer of acrylamid with methylene-bis-acrylamide as a linking agent assigned for lens manufacture. Said hydrogel contains 11% wt of copolymer (with acrylamide to methylene-bis-acrylamide mass ratio 100:2,26) and 89 wt per cent of physio-

logical salt solution.

Method of producing such a hydrogel described in the patent mentioned (GB No. 2114578) consists in copolymerization of acrylamide and methylene-bis-acrylamide as a linking agent in physiological salt solution in the presence of copolymerization initiators (tetramethylethylenediamine is one of them) with subsequent washing out of unreacted monomers from the end product. Copolymerization is conducted in a single stage at room temperature.

However, hydrogel thus obtained is unfit for soft tissue plastics because of its high consistence. Besides, due to a single stage process, this gel contains a lot of monomers and free radicals causing negative tissue response of organism.

Biocompatible hydrogel described in Claim EP No 742022 contains 3.5 to 9.0% wt of cross-linked copolymer of acrylamide with methylene-bis-acrylamide as a linking agent in aqueous medium.

Method of producing this hydrogel described in the Claim consists in acrylamide with methylene-bis-acrylamide copolymerization in aqueous medium in the presence of peroxide initiators. Reaction mass is maintained at room temperature for 20 minutes for cross-linking a polymer. Copolymerization process in this case is also a single-stage one, mixture of persulphate and tetramethylethylenediamine being used as peroxide initiator of copolymerization. A pyrogenic water or sodium chloride solution is taken as aqueous medium. Thus obtained hydrogel has inadequate degree of cross-linking due to low copolymerization process temperature and its single-stageness. It results in a rapid intergrowth of conjunctive tissue to the implanted gel and in its rapid shrinkage and resorption (see A.B.Shekhter et al "Injectible hydrophilic polyacrylamide gel Formacryl and tissue response to its Implantation" *Annals of Anaplastic, Reconstructive and Aesthetic Surgery*, 1997, No.2, p.19).

Besides, thus obtained hydrogel contains unbound tetramethylethylenediamine molecules, free NH₂ radicals and acrylamide monomers in the amount of 1.0-1.2 µg per 1g of gel (1.0-1.2 ppm). This can cause active asep-

tic inflammatory reaction at an early stage of gel implanting (see A.B.Shekhter et al "Injectible hydrophilic polyacrylamide gel Formacryl and tissue response to its Implantation", *Annals of Anaplastic, Reconstructive and Aesthetic Surgery*, 1997, No.2, p.19).

5 Biocompatible hydrogel described in RU Patent No.2127129 contains 1.0 to 8.0 %wt of cross-linked copolymer of acrylamide with methylene-bis-acrylamide in aqueous medium. Method of producing this hydrogel is described in the above mentioned patent RU No.2127129. It consists in copolymerization of acrylamide with methylene-bis-acrylamide (as a linking agent) in aqueous dispersing medium in the presence of peroxide initiator. Water subjected to electrolysis with pH of 9.0-9.5 is taken as aqueous dispersing medium. In this case copolymer's linking is carried out through the reaction blend incubation in two stages: at temperatures 20-90°C for 2-24 hours and then at 100-105°C for 2-4 hours.

10 15 Thus produced hydrogel does not contain unbound tetramethylethylene-diamine molecules, contains minimal quantity of free NH₂ radicals and acrylamide monomers in the amount of 0.6-0.8 µg per 1g of gel (1.0-1.2 ppm). Furthermore, after implanting this material to a patient's body it shrinks to 12-15% of the initial level thus reducing cosmetic effect of plastic surgery. In some cases additional material injection is needed.

Disclosure of Invention

20 25 An object of the present invention is to reduce resorption and shrinkage levels of a biocompatible hydrogel containing cross-linked acrylamide copolymer after its implanting to a patient's body by increasing monomers' cross-linking characterized by the presence of the following structural groups: (HC-NH-CH), (-CO-NH-CR-O-R), (-CO-NH-NH-CO-), (H-COR-NH-CR-O-R), (-CONH-R-NH-CO), where R = CH₃, CH₂, NH₂, C₂H₅. The task of diminishing tissue response of an organism to the implant by reducing a number 30 of free radicals and monomers in a hydrogel is also set.

The tasks set have been solved as follows: biocompatible hydrogel for

endoprosthetics containing cross-linked acrylamide copolymer with a linking agent and water according to the invention contains mixture of N, N'-methylene-bis-acrylamide and N, N'-ethylene-bis-acrylamide, with acrylamide to N, N'-methylene-bis-acrylamide and N, N'-ethylene-bis-acrylamide mass ratio being 95.0-99.5 : 0.4-4.5 : 0.1-0.5 respectively.

Said hydrogel can contain as a linking agent a mixture of N, N'-methylene-bis-acrylamide, N, N'-ethylene-bis-acrylamide and ethylene-bis-(oxyethylene nitril)-tetracetic oxide or ethylene-bis-(oxyethylene nitril)tetracetic acid with mass ratio of acrylamide, N, N'-methylene-bis-acrylamide, N,N'-ethylene-bis-acrylamide and ethylene-bis-(oxyethylene nitril)-tetraacetic oxide or ethylene-bis-(oxyethylene nitril)-tetracetic acid being 95.0-99.5 : 0.4-4.5 : 0.08-0.4 : 0.02-0.1.

Said hydrogel has pH 4.5-7.5.

Cross-linked copolymer makes up 2.0 to 15% of the total hydrogel by weight.

Hydrogel contains twice-distilled apyrogenic water as aqueous medium.

Additional feature of hydrogel is that the cross-linked copolymer has structural groups (HC-NH-CH), (-CO-NH-CR-O-R), (-CO-NH-NH-CO-), (H-COR-NH-CR-O-R), (-CONH-R-NH-CO), where R = CH₃, CH₂, NH₂, C₂H₅.

Biocompatible hydrogel's distinctive feature is that it is obtained by copolymerization of acrylamide, N, N'-methylene-bis-acrylamide and N,N'-ethylene-bis-acrylamide with their mass ratio 95.0-99.5 : 0.4-4.5 : 0.1-0.5 respectively, in aqueous dispersing medium in the presence of peroxide polymerization initiator with reaction mass incubation at 20-90°C for 2-24 hours and then at 115-130°C for 1-1.5 hours, with washing of semi-product in hot water at 90-100°C for 4-6 hours following the incubation at 20-90°C.

Said hydrogel can be also produced by copolymerization of acrylamide with N, N'-methylene-bis-acrylamide, N,N'-ethylene-bis-acrylamide and ethylene-bis-(oxyethylene nitril)-tetracetic oxide with their mass ratio 95.0-99.5 : 0.4-4.5 : 0.08-0.4 : 0.02-0.1 in aqueous medium in the presence of peroxide polymerization initiator with reaction mass incubation at 20-90°C for 2-24 hours and then at 115-130°C for 1-1.5 hours, with semi-product wash-

ing in hot water at 90-100°C for 4-6 hours which follows reaction mass incubation at 20-90°C.

The problems set are being solved by a method of producing biocompatible hydrogel by means of acrylamide and linking agent copolymerization in aqueous dispersive medium in the presence of peroxide copolymerization initiator with reaction mass incubation promoting copolymer's cross-linking in two stages. The first stage is carried out at 20-90°C for 2-24 hours, mixture of N, N'-methylene-bis-acrylamide and N, N'-ethylene-bis-acrylamide is taken as a linking agent, the second stage of incubation is held at 115-130°C for 1-1.5 h, the first incubation is followed by stock washing in hot water at 90-100°C during 4-6 h. In this case, mass ratio of acrylamide to N, N'-methylene-bis-acrylamide and N, N'-ethylene-bis-acrylamide is 95.0-99.5 : 0.4-4.5 : 0.1-0.5.

Method is also valid if mixture of N, N'-methylene-bis-acrylamide, N,N'-ethylene-bis-acrylamide and ethylene-bis-(oxyethylene nitril)-tetracetic oxide or ethylene-bis-(oxyethylene nitril)-tetracetic acid is used as a linking agent. In this case, mass ratio of acrylamide, N, N'-methylene-bis-acrylamide, N,N'-ethylene-bis-acrylamide and ethylene-bis-(oxyethylene nitril)-tetracetic oxide or ethylene-bis-(oxyethylene nitril)-tetracetic acid is 95.0-99.5 : 0.4-4.5 : 0.08-0.4 : 0.02-0.1.

For method's implementation aqueous dispersive medium and total amount of acrylamide and linking agents must be taken, with initial water to monomers' mixture ratio being 85.0-98.0 : 2.0-15.0 mass parts.

Stock washing is carried out at mass ratio of semi-product to water 1:8-10.

Hydrogen peroxide and/or ammonium persulfate are taken as polymerization initiators, in the amount not exceeding 0.3% wt.

Twice-distilled apyrogenic water is taken as aqueous medium.

Hydrogel material on the basis of copolymer of acrylamide and linking agents is known to constitute a three-dimensional net, in this particular case, of cross-linked copolymer of acrylamid, N, N'-methylene-bis-acrylamide (methylene-bis-acrylamide) and N, N'-ethylene-bis-acrylamide or cross-

linked copolymer of acrylamid, N, N'-methylene-bis-acrylamide (methylene-bis-acrylamide), N, N'-ethylene-bis-acrylamide and ethylene-bis-(oxyethylene nitril)-tetracetic oxide or ethylene-bis-(oxyethylene nitril)-tetracetic acid. Aqueous medium is retained within the net sells, certain undefined amount of unbound polymerization initiator is, in turn, retained within the aqueous medium. Another undefined amount of polymerization initiator directly incorporates into copolymer's structure (see M.N.Savitskaya, Y.D.Kholodova "Polyacrilamide", Technika, 1969, p.103) or is washed off the gel if washing occurs.

In addition, biologically active properties of such a gel to a considerable degree depend on cancellous structure of polymer, which, in turn, depends on polymer's synthesis conditions viz input components ratio, qualitative content of copolymerization initiators, which, by means of chemical and hydrogen bonds incorporate into copolymer's structure (through NH, CH, COOH, NH₂, CH₂ groups) and on temperature range of polymerization.

Essense of invention consists in experimental selection of conditions for biocompatible hydrogel production, which allowed reducing amount of unbound amides, free NH₂ radicals and unsaturated double bonds. In addition, linkage degree has been increased due to structural groups formation (HC-NH-CH), (-CO-NH-CR-O-R), (-CO-NH-NH-CO-), (H-COR-NH-CR-O-R), (-CONH-R-NH-CO), where R = CH₃, CH₂, NH₂, C₂H₅, and due to increasing amounts of cross-linking N-N bonds. All this allowed to ensure material's resistance to shrinkage, its high elasticity and tenacity, which, in turn, ensure high form stability of material at implanting. It was experimentally proved during hydrogel implanting to animals, rats and dogs in particular, with subsequent test of solid residual of hydrogel after 1, 3 and 6 month' residence of implant in animal bodies. Even after 6 month' residence loss of aqueous phase was found not to exceed 5% of its initial content in implant. Whereas loss of aqueous phase in implant obtained by method of RU patent No. 2127129 amounted to 10% at similar conditions.

Indirect evidence on the amount of unbound NH₂ radicals, monomers and unsaturated double bonds can be obtained by bromation level, IR spec-

troscopy and chromatomass- spectrometry data.

Bromation level of thus produced hydrogel does not exceed 2.0 mg Br/l and monomers content is not more than 0.4 ppm. Whereas assessment of known hydrogel according to RU patent No. 2127129 shows bromation level of 3.0 mg Br/l and monomers content of 0.6-0.8 ppm.

Hydrogel, IR spectrum and extract chromatogram, contains 96% of water phase and 4% of copolymer having 96 mass parts of acrylamide, 3.6 mass parts of N, N'-methylene-bis-acrylamide and 0.4 mass parts of N,N'-ethylene-bis-acrylamide in its content. It's pH=4.6, bromation level – 0.15 mg Br/l and it was obtained at incubation of initial blend in the presence of hydrogen peroxide and ammonium persulfate with 0.3% wt total at 60°C for 12 h and then at 120°C for one more hour.

As the spectrum shows, it lacks bands of 1620 cm^{-1} corresponding to deformation vibrations of NH_2 radicals, as well as 3200 cm^{-1} and 3600 cm^{-1} , corresponding to stretching vibrations of these radicals. It indicates that free NH_2 radicals content in polymer structure does not exceed 1% of all functional groups. As the chromatogram shows monomers content does not exceed 0.4 ppm.

Following substances are used for the method's implementation:

Acrylamide: $\text{C}_3\text{H}_5\text{NO}$, mol. wt is 71.08, white odourless crystal powder, melting point is 84,5°C, produced by Sigma Co. (USA);

$\text{N,N}'$ -methylene-bis-acrylamide: $\text{C}_7\text{H}_{10}\text{N}_2\text{O}_2$, mol.wt – 154,16, white odourless crystal powder, melting point is 185°C, produced by Sigma Co. (USA);

$\text{N,N}'$ - ethylene-bis-acrylamide produced by Aldrich Co. (USA);

Ethylene-bis-(oxyethylene nitril)-tetracetic oxide or ethylene-bis-(oxyethylene nitril)-tetracetic acid produced by Aldrich Co. (USA);

Ammonium persulfate: $(\text{NH}_4)_2\text{S}_2\text{O}_8$, mol. wt – 228.19, flat colourless crystals; decomposition point is 120°C; produced by Sigma Co. (USA);

All above-mentioned monomers must be fit for biological use and they must not need additional purification.

Twice-distilled apyrogenic water must be used (pH=5.6).

A method is carried out as follows:

Twice-distilled apyrogenic water (pH=5.6) is used for reaction mass preparation.

Aqueous solution of acrylamide and linking agents: N, N'-methylene-bis-acrylamide and N, N'-ethylene-bis-acrylamide (taken in mass ratio 95 : 0.4 : 0.1 e.g.) or N, N'-methylene-bis-acrylamide, N, N'-ethylene-bis-acrylamide and ethylene-bis-(oxyethylene nitril)-tetracetic oxide or ethylene-bis-(oxyethylene nitril)-tetracetic acid (taken in mass ratio 95 : 0.4 : 0.08 : 0.02 e.g.) is to be prepared, total amount of initial monomers in the solution is 2.0-15.0% (hydrogel of different consistency and elasticity can be obtained varying amount of initial monomers in the mass)

Polymerization initiators: hydrogen peroxide in the amount of 0.1-0.3 % wt or ammonium persulfate in the amount of 0.0006-0.03 % wt or their mixture in any ratio in the amount not exceeding their total maximum are put to the solution. By varying hydrogen peroxide and ammonium persulfate amounts a material of desirable pH is obtained. Finished reaction mass is filtrated through polymer bactericidal filters of F8273 brand with pore size of 0.45 mm CA/CN (producer – Sigma Co.(USA) and is incubated at 20-90°C for 2-24 h.

After the incubation semi-product of gel-like appearance must be washed with hot water. For that purpose it is placed for 4-6 h to a vessel with 90-100°C water, gel to water volume ratio being 1 : 8-10. Then the second stage of incubation at 115-130°C for 1.0-1.5 h is held. The gel obtained is packed in bottles of desirable volume and sterilised by autoclaving (at 120°C, p=1.2 atm) within 20 minutes.

The following hydrogel characteristics have been measured:

refractive index (by the technique described in "Physical Chemistry Workshop", M., 1974, pp.86-97);

pH (by the technique described in "Methodical Guideline For Sanitation Assessment of Rubber and Latex Goods of Medical Application", Moscow, 1988, pp.18,19);

bromation level (by the technique described in "Transactions of Guid-

ing Materials On Toxicology Study of Polymer Materials and Goods of Medical Application", M. Ministry of Health of the USSR, 1987., pp.27-29);

monomers content (by unpublished technique developed by the authors for evaluation of monomers content in water-containing polymers).

5 The claimed hydrogel has the following physicochemical characteristics:

appearance – colorless gel;

refractive index – 1.328-1.360;

density – 0.9-1.2 g/cm²;

10 pH – 4.5-7.5;

monomers content – ≤ 0,4 ppm;

bromation level – ≤ 2.0 mg Br/l.

Sanitary, chemical, toxicological and pathomorphological tests of the claimed biocompatible hydrogel were performed.

15 Tests were carried out in accordance with ISO 10993 Standard "Evaluation Of Biological Effect Of Medical Goods", "Transactions of Guiding Materials On Toxicology Study of Polymer Materials and Goods of Medical Application", "Admissible Quantities Of Migrations OF Chemical Substances Emissions From Polymers And Other Materials In Contact with Foodstaffs And Methods Of Their Evaluation" SanPin 42-122-42-40-86.

20 Sanitary and chemical tests showed that:

pH of water extract of hydrogel differed from a control test by 0.33, admissible difference being ±1;

25 migration of metals – Cu, Fe, Ni, Zn, Al, Ti, Ag to a hydrogel water extract, determined by atomic absorptive analysis was not indicated within the method's sensitivity value (0.02; 0.05; 0.05, 0.02; 0.005; 0.04 mg/l correspondingly), which is substantially lower than admissible levels for foodstaffs and drinking water;

30 sodium migration was 0.12 mg/l, admissible level in drinking water being 200 mg/l;

Toxicological tests showed that hydrogel extract did not display hemolytic effect 'in vitro' tests with isolated erythrocytes of rabbits. 0.04% hemol-

ysis occurred with admissible level of 2%.

Parenteral hydrogel injection (50 ml per 1 kg of body weight) in acute test with white mouses resulted in no deaths of animals or clinical intoxication signs: no difference occurred between behaviour, feeding up and hair condition of tested and control mouses.

Test mouses autopsy indicated that tissue at gel injection point, regional glands, viscuses (liver, kidneys, and spleen) were within the physiological norm and control.

No statistically reliable difference was found in body weight dynamics, clinical biochemical blood tests and viscus coefficients of tested and controlled animals after subcutaneous gel implanting for 2.5 months.

No sensitizing effect of gel was indicated during immunological diagnostic mast cell degranulation reaction (MCDR).

Microcore test on marrow substances indicated no mutagenic effect of the hydrogel. Histologic study of hydrogel implanting area and viscuses (liver, kidneys, spleen, testicles) showed feebly marked signs of tissue response to hydrogel only during first days after implanting and lack of dystrophic and necrotic changes in organs.

Tissue response to the claimed hydrogel implantation was studies during experimental and morphological and clinical morphological trials (see A.B.Shekhter et al "Injectible hydrophilic polyacrylamide gel Formacryl and tissue response to its Implantation", *Annals of Anaplastic, Reconstructive and Aesthetic Surgery*, 1997, No.2, p.11-21).

For these tests hydrogel samples were taken containing 96% of aqueous phase and 4% of copolymer with mass ratio of acrylamid, N, N'-methylene-bis-acrylamide (methylene-bis-acrylamide) and N, N'-ethylene-bis-acrylamide (pH=4.6) of 96 : 3.6 : 0.4, with bromation level of 0.15 mg Br/l. Hydrogel was obtained by incubation of initial mass in the presence of hydrogen peroxide and ammonium persulfate in the total amount of 0.3% wt at 60°C for 12 h and then at 120°C for one more hour, with stock washing in hot water (t=90°C) for 4 h.

Test was carried out on 160 male rats of August line, 200 g in weight

and 10 dogs.

1 ml of hydrogel was injected to a rat subcutaneously (intramuscular). Terms of morphological studies were 3, 7, 14, 30 and 90 days.

Long periods of implantation (6-12 months) were studied on dogs after 5 subcutaneous injection of 15 ml of hydrogel.

Clinical morphological studies of tissues after the implantation of the same gel to 5 patients were carried out: 1 month after subcutaneous implantation to a face area, 1.5 and 3.5 months after intramuscular implantation to shanks and 6 and 6.5 months after implantation for increasing mammoplasty 10 by filling a fibrous capsular with a gel after silicon prosthesis removal.

For histology studies tissue blocks were fixed in 96° alcohol or neutral 15 formalin and put in paraffin. Sections were colored with hematoxiline-eosin, picrofuxin by Van Gizon, silvering by Gomory to study fibrous components, with toluidine blue for acid glycosaminoglycanes. PAS response to glycogen and glycoproteins as well as Brashe response to RNA were studied.

Morphological study of body tissue response to the implantation of the claimed hydrogel carried out on rats showed that it was minimal. It was limited at early stages (3-7 days after the implantation) to a weak lympho- 20 macrophagal infiltration with isolated neutrophils and flabby tissue edema, which indicated a minimum inflammatory response. After 3 days' proliferation of fibroblasts in a narrow zone surrounding implant occurred, and a very thin conjunctive tissue capsular was formed by the 7th day which consisted 25 of fibroblasts and thin collagen fibers. This capsular was covered inside with nearly perpetual macrophage layer adjoining the implant. After 14 days the capsular got more distinctive but still remained thin and flocculent. In the capsular thickness as well as in the zone between the capsular and cellular 30 tissue small fragments of hydrogel surrounded by macrophages and isolated giant multinucleous cells can be seen. 1-3 months after the implantation the capsular is still thin. It consists of mature conjunctive tissue with reduced number of fibroblasts, the remaining cells have lesser RNA content. There are hydrogel fragments in the transcapsular area which are resorbed by macrophages. Interior surface of the capsular is still coated with

macrophages .

Tissue response at later terms (6, 9 and 12 months) was studied by subcutaneous implantation of gel to dogs. In a vicinity of a very thin and dense conjunctive tissue capsular a narrow lysis zone of the hydrogel can be seen. The hydrogel is resorbed by macrophages and germinated with fibroblast bars in that zone. There was no deep infiltration of cells into hydrogel neither in dog nor in rat experiments and that is the cause of its durable stability. Signs of lime sediments in the hydrogel can be found neither in dogs nor in rats. Dystrophic cell changes in tissue surrounding the implant, which could indicate toxic effect of the hydrogel, are not found.

Clinic and morphology observations held 1 month after injection of 90 ml of hydrogel for dermatensy of scin-fatty shred on a face with the purpose of further cicatrix plastics showed that a very thin and flocculent conjunctive tissue capsule is formed on a hydrogel – tissue boundary. That capsule consists of just a few layers of collagen fibres and fibroblasts. Cell lymphomacrophagal infiltration is minimal. Tissue vacuoles replacing resorbed gel can be seen in some spots outside the capsule. Feebly marked macrophagal and giant cell response is indicated there. Similar results were obtained in two cases of observations of contour plastics of shin soft tissue by the implantation of the claimed hydrogel. 1.5 and 3.5 months after the implantation hydrogel remained mostly homogeneous and conjunctive tissue germinated only near the capsular. In morphological study of biopsy taken 6.5 months after injection of 200 ml of the claimed hydrogel replacing silicone prosthesis in residuary fibros capsular cavity.

The tissue response to the gel is feebly marked. "Old" fibros capsular is in backward development in nearly all parts. The implant is surrounded by a thin conjunctive tissue capsular having no interior myofibroplastic layer which takes place in case of silicone prosthesis. In some spots, small lympho-macrophagal infiltrates without inflammatory neutrophilic response can be seen in a "new" capsular. Capsular's vasa are not numerous; there are no dystrophic changes and lime salt deposites in the capsular.

A superficial germination of thin conjunctive tissue bars (fibroblasts,

macrophags and thin immature collagen fibers) into the hydrogel can be seen in a vicinity of the capsular. Some macrophages have a big foamy cytoplasm (active phagocytosis). Conjunctive tissue bars divide gel around the capsular into fragments. Gel retained homogeneity in some fragments and obtained fine-meshed or fibrillar structure in some other fragments. Only isolated cell elements (macrophages) are seen inside remote from the capsular gel's parts.

Histology study results obtained in a long-term dynamics on animals as well as on biopsy clinic material indicate high biological compatibility of the hydrogel claimed. A very weak and rapidly disappearing inflammatory response was observed short time after the injection of claimed hydrogel into a body, fibroblastic response was weak and slow, late formation of a capsula took place and it remained thin for the whole period of observation.

It is significant that there was no deep invasion of macrophages and microphages into the hydrogel. It proves its stability to resorption in organism. At the same time hydrogel does not reduce functional activity of cells and does not cause their dystrophy. This proves lack of migration of toxic substanses from gel to tissue. There is no calcification of hydrogel and surrounding tissues.

Thus, the biologically compatible hydrogel claimed causes no tissue response, no organism sensitization, it is not mutagenic, it causes no dystrophic and necrotic changes and can be used for endoprosthetics and contour soft tissue plastics.

Here are examples of claimed method's realisation and use of hydrogel produced for soft tissue plastics.

EXAMPLE 1.

To obtain hydrogel, 19 g of acrylamide, 0.9 g of N,N'-methylene-bis-acrylamide and 0.1 g of N,N'-ethylene-bis-acrylamide suitable for biological application dissolved in 400 ml of twice-distilled apyrogenic water with a pH of 5.6. 0.04 g of ammonium persufate and 2 ml of 30% hydrogen peroxide were added to the solution. The mixture obtained was filtrated through polymer bactericidal filter of F8273 brand with pore size 0.45 mm CA/CN (producer - Sigma Co.(USA) and placed in the vessel which was put to a water

bath for incubation at 30°C for 22 hours. The gel like semi-product was washed in hot water with water to gel ratio 10:1 at temperature of 90°C for 4 hours and was further incubated for 1 hour at temperature not exceeding 125°C.

5 The hydrogel obtained was sterilised by autoclaving (at temperature of 120°C, pressure of 1.2 atm) within 20 minutes.

The produced material had the following physicochemical characteristics:

Appearance – colorless gel;

10 Refractive index – 1.348;

pH – 5,4;

Density – 1.0 g/cm²;

Monomers content – 0.2 ppm;

Bromation level – 0.1 (mg Br per 1L);

15 The hydrogel produced was injected to a female patient L-ya, aged 55, to replace a silicone prosthesis which had been used for primary mammoplasty 8 years before and caused coarse fibrosis of both mammals. The patient was being seen for 8 months' post-operative period with monthly examinations. Fibrosis recurrence was not detected. Positive result was obtained: 20 mammals' shape and size fit patient's constitution, their elasticity was typical for tissue of healthy mammal.

EXAMPLE 2

To obtain hydrogel, 24.5 g of acrylamide, 0.375 g of N,N'-methylene-bis-acrylamide, 0.1 g of N,N'-ethylene-bis-acrylamide and 0.025 g of ethylene-bis-(oxyethylene nitril)-tetracetic oxide or ethylene-bis-(oxyethylene nitril)-tetracetic acid dissolved in 1000 ml of twice-distilled apyrogenic water with a pH of 5.6. 2.5 g of ammonium persulfate was added to the solution. The mixture obtained was filtrated as described in example 1 and put to incubation at 80°C for 2 hours. The gel was washed in hot water at of 100°C for 5.5 hours and was further incubated for 1.5 hours at temperature of 125°C.

The hydrogel obtained was sterilised as described in example 1.

The produced material had the following physicochemical characteristics:

Appearance – colorless gel;
Refractive index – 1.344;
pH – 6,8;
Density – 1.0 g/cm²;
Acrylamide monomers content – absence;
Monomers content – 0.32 ppm;
Bromation level – 0.15 (mg Br per 1L).

The hydrogel obtained was used for subcutaneous tissue plasty for face wrinkles removal. The hydrogel was injected to a female patient S., aged 47. The patient was being seen for 12 months' post-operative period with periodic examinations of every 3 months. Inflammatory and allergic effects were not detected. Desirable cosmetic effect was obtained.

EXAMPLE 3

To obtain hydrogel, 77.6 g of acrylamide, 2.08 g of N,N'-methylene-bis-acrylamide and 0.32 g of N,N'-ethylene-bis-acrylamide dissolved in 1000 ml of twice-distilled apyrogenic water with a pH of 5.6. Then 0.03 g of ammonium persulfate was added to the solution. The mixture obtained was filtrated as described in example 1 and put to incubation at 60°C for 12 hours. The gel was washed in hot water at 100°C for 4.5 hours and was further incubated for 1.5 hours at temperature of 120°C.

The hydrogel obtained was sterilised as described in example 1.

The produced material had the following physicochemical characteristics:

Appearance – colorless gel;
Refractive index – 1.352;
pH – 5.2;
Density – 1.2 g/cm²;
Monomers content – 0.2 ppm;
Bromation level – 0.05 (mg Br per 1L).

The hydrogel obtained was used for sural muscle plasty. 150 g of hy-

drogel was implanted into each sural muscle of a patient S., aged 47. The patient was being seen for 12 months' post-operative period with periodic examinations of every 3 months. Inflammatory and allergic effects were not detected. Desirable cosmetic effect was obtained.

5 EXAMPLE 4

To obtain hydrogel, 23.75 g of acrylamide, 1.075 g of N, N'-methylene-bis-acrylamide, 0.045 g of N,N'-ethylene-bis-acrylamide and 0.005 g of ethylene-bis-(oxyethylene nitril)-tetracetic oxide or ethylene-bis-(oxyethylene nitril)-tetracetic acid dissolved in 400 ml of twice-distilled apyrogenic water with a pH of 5.6. Then 0.02 g of ammonium persulfate and 1 ml of 30% hydrogen peroxide was added to the solution. The mixture obtained was filtrated as described in example 1 and put to incubation at 50°C for 16 hours. The gel was washed in hot water at of 100°C for 4.0 hours and was further incubated for 1.5 hours at temperature of 130°C.

15 The hydrogel obtained was sterilised as described in example 1.

The produced material had the following physicochemical characteristics:

Appearance – colorless gel;

Refractive index – 1.348;

20 pH – 4.8;

Density – 1.0 g/cm²;

Monomers content – 0.3 ppm;

Bromation level – 0.12 (mg Br per 1L).

25 The hydrogel produced was injected to a female patient L-ya, aged 26, to replace a silicone prosthesis which had been used for primary mammoplasty 3 years before and caused fibrosis of both mammals 7 months after the operation. Surgery was performed to remove silicone prosthesis with open capsulotomy and delayed injection of 180 g of hydrogel to each mammal. 3 months later another 100 g of the same hydrogel was injected to each mammal. The patient was being seen for 7 months' post-operative period with once-every-two-months examinations. Fibrosis recurrence was not detected. As a result of surgery mammals' shape and size fit patient's constitu-

tion, their elasticity was typical for tissue of healthy mammal. A desirable cosmetic effect was obtained.

Results of the use of the claimed biocompatible hydrogel were published in the following papers: "Polyacrylamide gel in aesthetic surgery" (see 5 *Annals of Anaplastic, Reconstructive and Aesthetic Surgery*, 1997, No.2, p.7-9), A.I.Nerobeev et al "Experience with the use of polyacrylamide gel for contour plastics of soft tissues" (see the same as above, p.22-29), G.I.Lukomsky et al "Formacryl for mammoplasty and treatment of capsular fibroses" (see the same as above, pp.30-34), M.A.Sulamanidze et al "Subcutaneous dissection and liquid-gel dermotension" (see the same as above, pp.35-38), 10 "Capsular Fibrosis and its Treatment after Mammoplasty with Silicone Endoprostheses" (see the same as above, pp.75-87).

Thus, examples given prove possibility of producing biocompatible hydrogel and of using it for plastics of soft tissues. Such a hydrogel can also 15 be used as a filling agent for endoprostheses comprising a cover and a filling agent.

INTERNATIONAL SEARCH REPORT

Int'l Application No
PCT/IT 99/00428

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61L27/52 A61L31/14 A61L27/16 A61L31/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61L A61K C08F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, FSTA, COMPENDEX, INSPEC, BIOSIS, CHEM ABS Data, EMBASE, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>DATABASE WPI Section Ch, Week 200025 Derwent Publications Ltd., London, GB; Class A14, AN 2000-291292 XP002146496 & RU 2 127 129 C (LOPATIN V V), 10 March 1999 (1999-03-10) cited in the application abstract</p> <p>—</p> <p>—/—</p>	<p>1-4, 6, 8-12, 14, 16, 17, 20, 23, 26</p>

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the International search

4 September 2000

Date of mailing of the International search report

21/09/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl.
Fax: (+31-70) 340-3016

Authorized officer

Menidjel, R

INTERNATIONAL SEARCH REPORT

Intell. App. No.
PCT/IT 99/00428

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	OPPERMANN W., SCHRÖDER U.P., LINDEMANN B.: "Influence of the Cross-Linker Reactivity on the Formation of Inhomogeneities in Hydrogels" MACROMOLECULES, vol. 30, 1997, pages 4073-4077, XP002146495 abstract page 4073, left-hand column, paragraph 1 -right-hand column, paragraph 1 page 4074, left-hand column, paragraph 2 -right-hand column, paragraph 2 table 1	1-4, 6, 8-12, 14, 16, 17, 20, 23, 26
A	US 4 074 039 A (LIM DRAHOSLAV ET AL) 14 February 1978 (1978-02-14) abstract column 2, line 14 - line 34 column 2, line 56 -column 3, line 14	1-4, 6, 8-12, 14, 16, 17, 20, 23, 26
A	EP 0 742 022 A (INTERFALL MVP) 13 November 1996 (1996-11-13) abstract page 4, line 12 - line 33 page 4, line 56 -page 5, line 15 table 1	1-6, 8-14, 16, 17, 20, 21, 23, 26, 27
A	DATABASE WPI Section Ch, Week 200015 Derwent Publications Ltd., London, GB; Class A14, AN 2000-170073 XP002146497 & RU 2 122 438 C (KOSMOGEL CO), 27 November 1998 (1998-11-27) abstract	1-4, 6, 8-14, 16-18, 20, 22-24, 26, 28

95.0-99.5 : 0,4-4,5 : 0.1-0.5.

19. The method of claim 17 wherein semi-product washing is carried out at semi-product to water mass ratio of 1:8-10.

20. The method of claim 17 wherein hydrogen peroxide and/or ammonium persulfate are used as polymerization initiators; their content does not exceed 0.3% wt.

21. The method of claim 17 wherein twice-distilled apyrogenic water is used as aqueous medium.

22. The method of claim 17 wherein aqueous medium and mixture of acrylamide, N, N'-methylene-bis-acrylamide and N, N'-ethylene-bis-acrylamide are taken in initial mass of water to monomers mixture of 85.0-98.0 : 2.0-15.0 wt parts.

23. A method of producing biocompatible hydrogel by copolymerization of acrylamide with a linking agent in aqueous medium in the presence of peroxide polymerization initiator, with two-staged incubation of reaction mass, the first stage being held at temperature of 20-90°C for 2-24 hours, wherein mixture of N, N'-methylene-bis-acrylamide and N, N'-ethylene-bis-acrylamide and ethylene-bis-(oxyethylene nitril)-tetracetic oxide or ethylene-bis-(oxyethylene nitril)-tetracetic acid is used as the linking agent; the second stage of incubation is held at 115-130°C within 1-1.5 hours; the first incubation stage is followed by semi-product washing in hot water at 90-100°C for 4-6 hours.

24. The method of claim 23 wherein acrylamide, N, N'-methylene-bis-acrylamide, N, N'-ethylene-bis-acrylamide and ethylene-bis-(oxyethylene nitril)-tetracetic oxide or ethylene-bis-(oxyethylene nitril)-tetracetic acid are taken with mass ratio of 95.0-99.5 : 0,4-4,5 : 0.08-0.4 : 0.02-0.1.

25. The method of claim 23 wherein semi-product washing is carried out at semi-product to water mass ratio of 1:8-10.

26. The method of claim 23 wherein hydrogen peroxide and/or ammonium persulfate are used as polymerization initiators; their content does not exceed 0.3% wt.

27. The method of claim 23 wherein twice-distilled apyrogenic water is

used as aqueous medium.

28. The method of claim 23 wherein water medium and mixture of acrylamide, N, N'-methylene-bis-acrylamide, N, N'-ethylene-bis-acrylamide and ethylene-bis-(oxyethylene nitril)-tetracetic oxide or ethylene-bis-(oxyethylene nitril)-tetracetic acid are taken in initial water to monomers mixture ratio of 85.0-98.0 : 2.0-15.0 wt parts.

Claims

1. Biologically compatible hydrogel for endoprosthetics containing cross-linked copolymer of acrylamide with a linking agent and water wherein said linking agent is a mixture of N,N'-methylene-bis-acrylamide and N,N'-ethylene-bis-acrylamide, with acrylamide to N,N'-methylene-bis-acrylamide and N,N'-ethylene-bis-acrylamide mass ratio being 95.0-99.5 : 0.4-4.5 : 0.1-0.5 respectively.
2. The hydrogel of claim 1 wherein said cross-linked copolymer contains structural groups (HC-NH-CH), (-CO-NH-CR-O-R), (-CO-NH-NH-CO-), (H-COR-NH-CR-O-R), (-CONH-R-NH-CO), where R = CH₃, CH₂, NH₂, C₂H₅.
3. The hydrogel of claim 1 wherein its pH is 4.5-7.5.
4. The hydrogel of claim 1 wherein said cross-linked copolymer comprises 2 to 15% of total hydrogel weight.
5. The hydrogel of claim 1 wherein it contains twice-distilled apyrogenic water as aqueous medium.
6. The biocompatible hydrogel of claim 1 wherein it is obtained by copolymerization of acrylamide with N, N'-methylene-bis-acrylamide and N,N'-ethylene-bis-acrylamide, their mass ratio is 95.0-99.5 : 0.4-4.5 : 0.1-0.5 in aqueous medium in the presence of peroxide polymerization initiator with reaction mass incubation at 20-90°C for 2-24 hours and then at 115-130°C for 1-1.5 hours, with semi-product washing in hot water at 90-100°C for 4-6 hours which follows reaction mass incubation at 20-90°C.
7. The hydrogel of claim 6 wherein semi-product washing is carried out at semi-product to water mass ratio of 1:8-10.
8. The hydrogel of claim 6 wherein hydrogen peroxide and/or ammonium persulfate are used as polymerization initiators; their content does not exceed 0.3% wt.
9. The biologically compatible hydrogel for endoprosthetics containing cross-linked copolymer of acrylamide with a linking agent and water wherein it contains as a linking agent mixture of acrylamide with N, N'-methylene-bis-acrylamide, N, N'-ethylene-bis-acrylamide and ethylene-bis-(oxyethylene nitril)-tetracetic oxide or ethylene-bis-(oxyethylene nitril)-tetracetic acid with

their mass ratio of 95.0-99.5 : 0,4-4,5 : 0.08-0.4 : 0.02-0.1.

10. The hydrogel of claim 9 wherein said cross-linked copolymer contains structural groups (HC-NH-CH), (-CO-NH-CR-O-R), (-CO-NH-NH-CO-), (H-COR-NH-CR-O-R), (-CONH-R-NH-CO), where R = CH₃, CH₂, NH₂, C₂H₅.

5 11. The hydrogel of claim 9 wherein its pH is 4.5-7.5.

12. The hydrogel of claim 9 wherein said cross-linked copolymer comprises 2 to 15% of total hydrogel weight.

13. The hydrogel of claim 9 wherein it contains twice-distilled apyrogenic water as aqueous medium.

10 14. The hydrogel of claim 9 wherein it is obtained by copolymerization of acrylamide, N, N'-methylene-bis-acrylamide, N, N'-ethylene-bis-acrylamide and ethylene-bis-(oxyethylene nitril)-tetracetic oxide or ethylene-bis-(oxyethylene nitril)-tetracetic acid with their mass ratio of 95.0-99.5 : 0,4-4,5 : 0.08-0.4 : 0.02-0.1 in aqueous medium in the presence of peroxide polymerization initiator with reaction mass incubation at 20-90°C for 2-24 hours and then at 115-130°C for 1-1.5 hours, with semi-product washing in hot water at 90-100°C for 4-6 hours which follows reaction mass incubation at 20-90°C.

15 15. The hydrogel of claim 14 wherein semi-product washing is carried out at semi-product to water mass ratio of 1:8-10.

20 16. The hydrogel of claim 14 wherein hydrogen peroxide and/or ammonium persulfate are used as polymerization initiators; their content does not exceed 0.3% wt.

25 17. A method of producing biocompatible hydrogel by copolymerization of acrilamide with a linking agent in aqueous medium in the presence of peroxide polymerization initiator, with two-staged incubation of reaction mass, the first stage being held at temperature of 20-90°C for 2-24 hours, wherein mixture of N, N'-methylene-bis-acrylamide and N, N'-ethylene-bis-acrylamide is used as the linking agent; the second stage of incubation is held at 115-130°C within 1-1.5 hours; the first incubation stage is followed by semi-product washing in hot water at 90-100°C for 4-6 hours.

30 18. The method of claim 17 wherein acrylamide, N, N'-methylene-bis-acrylamide and N, N'-ethylene-bis-acrylamide are taken in mass ratio of

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/IT 99/00428

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
RU 2127129	C	10-03-1999	NONE		
US 4074039	A	14-02-1978	CA	1002677 A	28-12-1976
			DE	2112487 A	14-10-1971
			FR	2085122 A	17-12-1971
			GB	1319751 A	06-06-1973
EP 0742022	A	13-11-1996	AT	190508 T	15-04-2000
			AU	696773 B	17-09-1998
			AU	7794094 A	07-03-1996
			BG	101251 A	30-09-1997
			BR	9408609 A	23-12-1997
			CA	2195957 A	22-02-1996
			CZ	9700224 A	16-07-1997
			DE	69423520 D	20-04-2000
			ES	2145841 T	16-07-2000
			FI	970315 A	24-01-1997
			JP	10503950 T	14-04-1998
			NO	970330 A	10-02-1997
			RU	2067873 C	20-10-1996
			WO	9604943 A	22-02-1996
			US	5798096 A	25-08-1998
			PL	318764 A	07-07-1997
RU 2122438	C	27-11-1998	NONE		

THIS PAGE BLANK (USPTO)